

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Richard B. Roth *et al.*

Serial No.: 10/723,518

Filing Date: November 25, 2003

Title: METHODS FOR IDENTIFYING RISK
OF BREAST CANCER AND
TREATMENTS THEREOF

Examiner: Jehanne Souaya Sitton

Group Art Unit: 1634

Conf. No.: 8937

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. CHARLES CANTOR UNDER 37 C.F.R. 1.132

Dear Sir:

I, Charles Cantor, declare as follows:

1. I have studied the human genome for a large part of my scientific career and a copy of my *curriculum vitae* is attached. I was Director of the Human Genome Center Project of the Department of Energy at Lawrence Berkeley Laboratory, and published the first textbook on genomics entitled *Genomics: The Science and Technology of the Human Genome Project*. I also was the Chair and Professor of the Department of Biomedical Engineering and Biophysics, and Director of the Center for Advanced Biotechnology, at Boston University. Before taking that position, I held professorship positions at Columbia University and the University of California, Berkeley. I have been the Chief Scientific Officer and Chairman of the Scientific Advisory Board of Sequenom, Inc. since 1998, and in May 2000 was appointed to the company's Board of Directors. I oversaw studies carried

out by the inventors that led to the scientific discoveries presented in the above-identified patent application.

2. I have reviewed the patent application and the rejection in the Office action dated August 30, 2006 based on a perceived lack of written description. I understand this perception may be addressed and overcome by showing the inventors had possession of the claimed methods at the time the patent application was filed. Below, I describe (i) methodology and discoveries presented in the patent application, (ii) genetic principles forming the basis for these discoveries, (iii) identification of another disease-associated locus using similar methodology, (iv) publicly available database information in support of the reported discoveries, and (v) other experimental evidence substantiating the discoveries. The data and methodology presented in the patent application and supporting evidence show the inventors had possession of the claimed methods at the time of filing.

3. Presented in the patent application is a genomic study in which many single nucleotide polymorphism (SNP) positions, spaced throughout the entire human genome, were typed in two populations: a breast cancer population and a "healthy" control population. Certain variants occurring with a significantly high frequency in the cancer population were identified as markers residing in candidate loci associated with the disease. The inventors then verified that additional SNPs proximal to the incident marker polymorphism in each locus were significantly associated with the disease. Regions that contained multiple disease-associated polymorphisms were verified as being significantly associated with breast cancer. One region identified by this method was a region encoding the RAD21 protein, and the inventors typed 58 polymorphic sites in this region. Of these, several polymorphisms were significantly associated with breast cancer, and they clustered in a sub-region of the RAD21 locus. This sub-region, or "hot zone," is the region specified in amended claim 1, and illustrated in Figure 4 of the patent application. Thus, the inventors identified that the RAD21 region was significantly associated with breast cancer by (i) identifying a locus containing an incident polymorphic marker associated with the

disease in a genome-wide scan, and (ii) verifying multiple polymorphic sites proximal to the incident marker in the locus also were associated with the disease.

4. Identifying a disease-associated region by this methodology is supported by accepted genetic principles. The concept of linkage disequilibrium in genetics embodies the phenomenon that a disease-associated region in the human genome contains a cluster of polymorphisms associated with a disease state. Specifically,

markers very close to the disease gene will tend, more likely than average, to retain the haplotype of the original chromosome because, as the distance to the disease gene shrinks, it becomes less likely that recombination events will have occurred in this particular region.

From Cantor & Smith, *Genomics: The Science and Technology Behind the Human Genome Project*, 1999, John Wiley & Sons, Inc., New York, page 192. Thus, identifying multiple polymorphisms associated with a disease state within a region identifies the region as associated with the disease state. Accordingly, the inventors identified the claimed region as being associated with breast cancer when they verified multiple polymorphic variants in the region were significantly associated with the disease.

5. Identifying a disease-associated region by this methodology is supported by the work of other researchers. Researches using similar methods independently located a complement factor H (CFH) gene region as being significantly associated with age-related macular degeneration (AMD). Klein *et al.*, like the inventors of the present patent application, screened SNPs spaced across the entire human genome in disease and control populations, and identified polymorphisms significantly associated with the disease¹. Klein *et al.* then sequenced exons in the locus containing the associated SNPs and identified proximal polymorphisms associated with the disease. Edwards *et al.*, screened proximal polymorphisms in the CFH region and identified a sub-region particularly associated with the disease². Edwards *et al.* illustrated the disease-associated region

using a plot similar to Figure 4 in the present patent application (Figure 1 in Edwards *et al.*). Haines *et al.* also identified the same CFH gene region as being associated with AMD from a 261 kilobase pair region containing a haplotype associated with AMD³. Like Edwards *et al.*, Haines *et al.* illustrated the disease-associated region using a plot similar to Figure 4 in the present patent application (Figure 2 in Haines *et al.*). Hageman *et al.* and Zarepari *et al.* further confirmed the association of the CFH region with AMD^{4,5}.

6. I would like to point out that the inventors typed a significant number of polymorphisms in the RAD21 region in the process of determining that the region was associated with breast cancer. The following table shows, according to the RAD21 region analyzed and the sub-region claimed, (i) the number of polymorphisms the inventors analyzed, (ii) the number of polymorphisms in the HapMap database, (iii) the number of polymorphisms analyzed by the inventors overlapping with the HapMap database polymorphisms, (iv) the number of polymorphisms in the HapMap database having a minor allele frequency (MAF) of greater than 0.05, and (v) the number of polymorphisms analyzed by the inventors overlapping with the HapMap database polymorphisms having a MAF greater than 0.05.

Region	Inventor polymorphisms (IP)	HapMap polymorphisms (HP)	Overlap of IP and HP	HP with MAF >0.05 (HP>0.05)	Overlap of IP and HP>0.05
RAD21	58	182	41 (23%)	78	28 (34%)
Claimed RAD21 sub-region	16	39	12 (31%)	12	5 (42%)

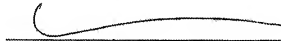
In the claimed sub-region, the inventors therefore analyzed 42% of the polymorphisms currently in the HapMap database having a MAF of greater than 0.05 at the time the present patent application was filed (i.e., November 25, 2003), which was more than three-and-a-half years ago. This degree of overlap with polymorphisms in the HapMap database is on par with, or better than, the overlap of polymorphisms Klein *et al.* analyzed for determining the association of CFH with AMD. Specifically, Klein *et al.* analyzed 13% of the CFH region SNPs in the HapMap database (analyzed 19 of 152 in the database;

paragraph spanning pages 386-387). The inventors therefore identified the claimed RAD21 sub-region association with breast cancer by analyzing a significant number of polymorphisms.

7. The inventors also collected biological data showing that the RAD21 region was associated with breast cancer. The inventors compared levels of mRNA encoded by the analyzed genomic region and found target mRNA levels were lower in normal, immortalized breast cell lines, while higher levels were identified in tumorigenic breast cancer cell lines (e.g., specification, paragraph 0239). Also, inhibiting the target region by RAD21-directed siRNA molecules significantly reduced proliferation of breast cancer cells (e.g., specification, paragraphs 0242 and 0243). Breast cancer cell lines treated with RAD21 specific siRNA molecules also were significantly more sensitive to chemotherapeutic agents than cell lines not treated with the siRNA molecules (e.g., specification, paragraph 0247). Thus, the inventors demonstrated the RAD21 region was significantly associated with breast cancer by the biological evidence presented in the patent application.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed in San Diego, California, on 25 July 2007.

A handwritten signature in black ink, consisting of a large, sweeping 'C' followed by a horizontal line.

Charles Cantor, Ph.D.

Documents cited:

1. Klein *et al.*, "Complement factor H polymorphism in age-related macular degeneration," *Science* 308: 385-389 (2005).
2. Edwards *et al.*, "Complement factor H polymorphism and age-related macular degeneration," *Science* 308: 421-424 (2005).
3. Haines *et al.*, "Complement factor H variant increases the risk of age-related macular degeneration," *Science* 308: 419-421 (2005).
4. Hageman *et al.*, "A common haplotypes in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration," *Proc. Natl. Acad. Sci.* 102(20): 7227-7232 (2005).
5. Zarepari *et al.*, "Strong association of the Y402H variant in complement factor H at 1q32 with susceptibility to age-related macular degeneration," *Am. J. Hum. Genet.* 77: 149-153 (2005).

Charles R. Cantor, PhD

Academic Qualifications: Columbia University, A.B., 1963. University of California, Berkeley, California US, Ph.D. 1966.

Academic Qualifications: 1966-69, Asst. Prof. of Chemistry, Columbia Univ.; 1969-72, Assoc. Prof. of Chemistry, joint appointment in Biological Sciences, Columbia Univ.; 1972-81, Prof. of Chemistry, joint appointment in Biological Sciences, Columbia Univ.; 1981-89, Prof. and Chair of Genetics and Development, College of Physicians and Surgeons, Columbia Univ., Deputy Dir. for Education, 1981-85, Comprehensive Cancer Ctr., and Deputy Dir. for Biotechnology, 1985-88, Comprehensive Cancer Ctr.; 1988-89, Higgins Prof. of Genetics and Development, Faculty of Medicine, Columbia Univ.; 1988-90, Dir., Human Genome Ctr., Lawrence Berkeley Laboratory; 1989-91, Sr. Biochemist, Cell and Molecular Biology Div., Lawrence Berkeley Laboratory; 1989-92, Prof. of Molecular Biology, Univ. of CA, Berkeley; 1990-92, Principal Scientist, Human Genome Project, U.S. Dept. of Energy; 1991-92, Sr. Biochemist, Chemical Biodynamics Div., Lawrence Berkeley Laboratory; 1992-pres., Prof. of Biomedical Engineering, Boston Univ.; 1992-pres., Dir., Ctr. for Advanced Biotechnology, Boston Univ.; 1994-pres., Prof., Pharmacology Dept., Boston Univ. Medical School; 1995-98, Chair, Dept. of Biomedical Engineering, Boston Univ.; 1998-pres., Chief Scientific Officer, Sequenom, Inc.; 2002, Founder, SelectX Pharmaceuticals, Inc.; 2005, Founder, Retrotype, Inc.

Present Positions: Boston University, Boston, MA USA, co-director of the Center for Advanced Biotechnology and professor of Biomedical Engineering. Sequenom, Inc., San Diego, CA USA, Chief Scientific Officer, founder and member, Board of Directors.

Research Work and Funding: Large projects including Director of the Human Genome Center of the Department of Energy at Lawrence Berkeley Laboratory. 35 years of continuous research funding from government and private sources.

Awards and Honors:

1969-71, Fellow of the Alfred P. Sloan Foundation; 1972, Fresenius Award in Chemistry; 1973-74, Guggenheim Fellow; 1975-76, Fairchild Distinguished Visiting Scholar, CA Inst. of Technology; 1978, Eli Lilly Award in Biological Chemistry; 1981, Fellow of the Amer. Assoc. for the Advancement of Science; 1985, Outstanding Investigator Grant, Natl. Cancer Inst.; 1988, Biochemical Analysis Prize of the German Society of Clinical Chemistry; 1988, Member of the Natl. Acad. of Sciences; 1988, Member of the Amer. Acad. of Arts and Sciences; 1989, ISCO Award for Advances in Biochemical Instrumentation; 1990, Herbert A. Sober Award, Amer. Society for Biochemistry and Molecular Biology; 1990, Honorary Member, Japanese Biochemical Society; 1992, Fellow of the CA Acad. of Sciences; 1993, Fellow of the Biophysical Society; 2000, Emily M. Gray Award from the Biophysical Society; 2002, Chief Scientist of the Year, T Sector and BIOCOM; 2004, Ohio State University Human Cancer Genetics Program Commemorative Medal for Excellence in Research and Clinical Care

Selected Publications since 2004:

Smylie, K.J., Cantor, C.R., and Denissenko, M. 2004. *Analysis of Sequence Variations in Several Human Genes using Phosphoramidite Bond DNA Fragmentation and Chip-based MALDI-TOF*. Genome Research, 14, 134-141.

Karaoz, U., Murali, T.M., Letovsky, S., Zheng, Y., Ding, C., Cantor, C.R., and Kasif, S. 2004. *Whole Genome Annotation Using Evidence Integration in Functional Linkage Networks*. Proc. Nat. Acad. Sci. USA, 101, 9, 2888-2893.

Ding, C., Maier, E., Roscher, A.A., Braun, A., and Cantor, C.R. 2004. *Simultaneous Quantitative and Allele-specific Expression Analysis with Real Competitive PCR*. BMC Genetics 2004:5:8

Antonenko, Y.N., Rokitskaya, T.I., Kotova, E.A., Reznik, G.O., Sano, T., and Cantor, C.R. 2004. *Effect of Streptavidins with Varying Biotin Binding Affinities on the Properties of Biotinylated Gramicidin Channels*. Biochemistry 43, 4575-4582.

Kobayashi, H., Kaern, M., Araki, M., Chung, K., Gardner, T.S., Cantor, C.R., and Collins, J.J. 2004. *Programmable Cells: Interfacing Natural and Engineered Gene Networks*. Proc. Nat. Acad. Sci. USA, 101, 22, 8414-8419.

Isaacs, F.J., Dwyer, D.J., Ding, C., Pervouchine, D.D., Cantor, C.R., and Collins, J.J. 2004. *Engineered Riboregulators Enable Post-transcriptional Control of Gene Expression*. Nature Biotech., 22, 841-847.

Ding, C., Chiu, R.W.K., Lau, T.K., Leung, T.N., Chan, L.C., Chan, A.Y.Y., Charoenkwan, P., Ng, I.S.L., Law, H., Ma, E.S.K., Xu, X., Wanapirak, C., Sanguanersmsri, T., Liao, C., Tan Jin Ai, M., Chiu, D.H.K., Cantor, C.R., and Lo, Y.M. Dennis. 2004. *Mass Spectrometry Analysis of Single-nucleotide Differences in Circulating Nucleic Acids: Application to Noninvasive Prenatal Diagnosis*. Proc. Nat. Acad. Sci. USA, 101, 29, 10762-10767.

Nelson, M.R., Marnellos, G., Kammerer, S., Hoyal, C.R., Shi, M.M., Cantor, C.R., and Braun, A. 2004. *Large-scale Validation of Single Nucleotide Polymorphisms in Gene Regions*. Genome Research, 14, 1664-1668.

Zhang, L., Kasif, S., Cantor, C.R., and Broude, N.E. 2004. *GC/AT-content Spikes as Genomic Punctuation Marks*. Proc. Nat. Acad. Sci. USA, 101, 16855-16860.

Kammerer, S., Roth, R.B., Reneland, R., Marnellos, G., Hoyal, C.R., Markward, N.J., Ebner, F., Kiechle, M., Schwarz-Boeger, U., Griffiths, L.R., Ulbrich, G., Chrobok, K., Forster, G., Praetorius, G.M., Meyer, P., Rehbock, J., Cantor, C.R., Nelson, M.R., and Braun, A. 2004. *Large-Scale Association Study Identifies ICAM Gene Region as Breast and Prostate Cancer Susceptibility Locus*. Cancer Research, 64, 8906-8910.

Honisch, C., Raghunathan, A., Cantor, C.R., Palsson, B.O., and van den Boom, D. 2004. *High-throughput Mutation Detection Underlying Adaptive Evolution of Escherichia Coli-K12*. Genome Research, 13, 2495-2502.

- Cantor, C.R. and Nelson, M.R. 2005. *Haplotyping in Biomedicine – Practical Challenges*. Nature Biotech., 23, 21-22.
- Yanai, I., Yu, Y., Cantor, C.R., and Weng, Z. 2005. *An Avidin-like Domain That Does Not Bind Biotin is Adopted for Oligomerization by the Extracellular Mosaic Protein Fibropellin*. Protein Science, 14, 417-423.
- Kammerer, S., Roth, R.B., Hoyal, C.R., Reneland, R. Marnellos, G. Kiechle, M., Schwarz-Boeger, U., Griffiths, L.R., Ebner, F., Rehbock, J., Cantor, C.R., Nelson, M.R., and Braun, A. 2005. *Association of the NuMA Region on Chromosome 11q13 with Breast Cancer Susceptibility*. Proc. Nat. Acad. Sci. USA, 102, 2004-2009.
- Rachlin, J., Ding, C., Cantor, C.R., and Kasif, S. 2005. *MuPlex: Multi-Objective Multiplex PCR Assay Design*. Nucleic Acids Research, 33, 544-547.
- Jurinke, C., Denissenko, M.F., Oeth, P., Ehrich, M., van den Boom, D., and Cantor, C.R. 2005. *A single Nucleotide Polymorphism Based Approach for the Identification and Characterization of Gene Expression Modulation Using MassARRAY*. Mutation Research, 573, 83-95.
- Aslan, F.M., Yu, Y., Mohr, S.C., and Cantor, C.R. 2005. *Engineered Single-chain Dimeric Streptavidins with an Unexpected Strong Preference for Biotin-4-fluorescein*. Proc. Nat. Acad. Sci. USA, 102, 8507-8512.
- McCullough, R.M., Cantor, C.R., and Ding, C. 2005. *High-throughput Alternative Splicing Quantification by Primer Extension and Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*. Nucleic Acids Research, 33 (11), e99, 1-9.
- Rachlin, J., Ding, C., Cantor, C.R., and Kasif, S. 2005. *Computational Tradeoffs in Multiplex PCR Assay Design for SNP Genotyping*. BMC Genomics, 6, 102.
- Ehrich, M., Nelson, M.R., Stanssens, P., Zabeau, M., Liloglou, T., Xinarianos, G., Cantor, C.R., Field, J.K., and van den Boom, D. 2005. *Quantitative High-throughput Analysis of DNA Methylation Patterns by Base-Specific Cleavage and Mass Spectrometry*. Proc. Nat. Acad. Sci. USA, 102, 15785-15790.
- Moiseev, L., Unlu, M.S., Swan, A.K., Goldberg, B.B., and Cantor, C.R. 2006. *DNA Conformation on Surfaces Measured by Fluorescence Self-interference*. Proc. Nat. Acad. Sci. USA, 103, 2623-2628.
- Demidov, V., Dokholyan, N., Witte-Hoffman, C., Chalasani, P., Yiu, H-W., Ding, F., Yu, Y., Broude, N., and Cantor, C.R. 2006. *Fast Complementation of Split Fluorescent Protein Triggered by DNA Hybridization*. Proc. Nat. Acad. Sci. USA, 103, 2052-2056.
- Guido, N.J., Wang, X., Adalsteinsson, D., McMillen, D., Hasty, J., Cantor, C.R., Elston, T.C., and Collins, J.J. 2006. *A Bottom-up Approach to Gene Regulation*. Nature, 439, 856-860.
- Herring, C.D., Raghunathan, A., Honisch, C., Patel, T., Applebee, M. K., Joyce, A.R., Albert, T.J., Blattner, F.R., van den Boom, D., Cantor, C.R. and Palsson, B.O. 2006. *Comparative genome sequencing of Escherichia coli allows observation of bacterial evolution on a laboratory timescale*. Nat. Gen. 38, 12, 1406-1412.
- Alon, N., Asodi, V., Cantor, C., Kasif, S., Rachlin, J. 2006. *Multi-node Graphs: A framework for Multiplexed Biological Assays*. J. Comp. Biol. Dec: 13 (10):1659-72.
- Rachlin, J., Cohen, D.D., Cantor, C., and Kasif, S. 2006. *Biological context networks: a mosaic view of the interactome*. Molecular Systems Biology 2:66.
- Blake, W., Balazsi, G., Kohanski, M.A., Isaacs, F.J., Murphy, K.F., Kuang, Y., Cantor, C.R., Walt, D.R., Collins, J.J. 2006. *Phenotypic consequences of promoter-mediated transcriptional noise*. Molecular Cell 2006 24:853-865.
- Lo, Y.M.D., Tsui, N.B.Y., Chiu, R.W.K., Lau, T.K., Leung, T.N., Heung, M.M.S., Gerovassili, A., Jin, Y., Nicolaides, K.H., Cantor, C.R., and Ding, C. 2007. *Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection*. Nat. Med. 13, 218-223.
- Zhang, L., Kasif, S., Cantor, C.R. 2007. *Quantifying DNA-protein binding specificities by using oligonucleotides mass tags and mass spectrometry*. Proc. Nat. Acad. Sci. USA, 104, 3061-3066.
- Honisch, C., Chen, Y., Mortimer, C., Arnold, C., Schmidt, O., van den Boom, D., Cantor, C.R., Shah, H. N., Gharbia, S.E. 2007. *An automated comparative sequence analysis approach: Microbial typing by base-specific cleavage and MALDI-TOF mass spectrometry*. Proc. Nat. Acad. Sci. USA, in process.

Selected Recent Professional Affiliations and Service:

1976-pres., Series Editor, Advanced Textbooks in Chemistry, Springer-Verlag, New York; 1978-pres., Biophysical Society (Council Member, 1978-81); 1982-pres., Amer. Society of Biochemistry and Molecular Biology; 1984-pres., Nomenclature Commission of the Intl. Union of Biochemistry and Molecular Biology; 1985-pres., Scientific Advisory Council, Roswell Park Memorial Inst.; 1988-pres., Biomedical Advisory Committee, Pittsburgh Supercomputing Ctr.; 1988-pres., Cell and Membrane Transport Commission, Intl. Union of Pure and Applied Biophysics; 1988-pres., Member, Exec. Committee and Founding Council, Intl. Human Genome Organization (HUGO) and 1990-2006, Vice President; 1991-95, Chair, HUGO Human Genome Mapping Committee (HGMC); 1992-1996, President, HUGO Americas; 1988-pres., Editorial Board, *Current Opinion in Biotechnology*; 1992-97, Member, Board of Dir., Chair, Scientific Advisory Board, ATGC/AT Biochem, Inc.; 1992-02, Member, Advisory Board, Boston Univ. Journal of Science Technology and Law; 1994-pres., Co-chair, Biotechnology Advisory Committee, Fisher Scientific; 1996-pres., Member, Editorial Board, Biotechniques; 1996-97, Member, NRC Committee, "Bits of Power"; 1997-98, Member, DARPA Advisory Committee on Biological Warfare Defense; 1996-2000, Member, Advisory Board, Encyclopedia of Molecular Biology; 1996-00, Member, FASEB Consensus

Committee on Federal Funding, representing the Biophysical Society; 1997-2000, Quest Scholar, Quest Diagnostics, Inc., San Juan Capistrano, CA; 1998-2000, Member, Defense Intelligence Agency Bio 2020 Red Team, Washington, D.C.; 2000-pres., Adjunct Prof., Dept. of Bioengineering, UCSD; 2001-pres., Science Advisory Board, Brandeis Univ. School of Science; 2002, Advisory Committee Member, Stockholm Strategic Research Foundation; 2002-pres., Scientific Advisory Board, Buffalo Ctr. of Excellence in Bioinformatics; 2003-pres., Member, National Advisory Board, Boston University Research Center for Translational Genomics and Human Rights, Boston, MA; 2004-pres., Scientific Advisory Board, Joint Center for Structural Genomics, La Jolla, CA; 2004-pres., Scientific Advisory Board, Uppsala Bio-X, Uppsala, Sweden. Currently Board member or advisor for 20 or more companies including Fisher Scientific, US, Techno Ventures Management (Germany), Strand Genomics (India). 44 issued U.S. patents.